Research Article

Journal of Dental and Maxillofacial Surgery

Gender and Smoking Correlations of Surfactant Lipids and Proteins in the Saliva of Dental Patients

Shawn S Adibi^{1*}, Joseph L Alcorn², Kaori Ono² and Lenard M Lichtenberger²

¹Department of General Practice & Dental Public Health, University of Texas School of Dentistry at Houston, Houston, Texas 77054, USA

²The McGovern Medical School at UTHealth, 6431 Fannin, Suite 3.222, Houston, Texas 77030, USA

***Correspondence:** Shawn S Adibi, Department of General Practice & Dental Public Health, University of Texas School of Dentistry at Houston, TX 77054, USA, Tel: 713-486-4565; E-mail: Shawn.adibi@uth.tmc.edu

Received: September 21, 2018; Accepted: October 16, 2018; Published: October 22, 2018

Abstract

We sought to determine the effects of smoking on surfactant lipids and proteins in saliva. Levels of sphingomyelin (Sph) phosphatidylcholine (PC) and lyso-PC (LPC) were determined by thin layer chromatography. Levels of surfactant protein A (SP-A) were determined by western analysis using antibodies specific for SP-A. Significance of the results was determined by the student's *t*-test. The LPC/PC ratio had a tendency to be much higher in smokers compared to non-smokers. LPC levels were significantly higher in females smokers compared to male smokers. Additionally, levels of SP-A were significantly reduced in females smokers compared to non-smokers. Smoking alters surfactant protein and LPC/PC ratios in saliva. There is a significant difference in the effects in females compared to males. Findings suggest smoking alters the composition of saliva that may reduce protection of the oral cavity, which may explain why women smokers are at greater risk of developing oral mucositis.

Introduction

Oral mucositis (OM) is a condition associated with painful mouth ulcers and inflammation that occurs in many individuals, one of the unfortunate consequences of cancer therapies is the development of painful mouth sores associated with Xerostomia (also called "dry-mouth" by some). Dry mouth also is a common condition of patients with Sjögren's syndrome, and also is a side effect of: a number of medications (called xerogenics such as; anti-cholinergics, H-2 blockers anti-depressants and sympathetic agonists). Cancer patients who receive chemotherapy or radiation therapy for head and neck tumors, breast cancer or hematologic malignancies are at very high risk to develop long-term and severe oral mucositis. They are medically compromised and often with complex medical history. Dry mouth occurs more commonly in women (up to 30%), smokers and the elderly.

Some people have very few side effects from cancer therapy while others have many [1],[2]. There is evidence that saliva contains significant concentrations of Surface-Active Phospholipids (SAPLs), notably phosphatidylcholine (PC), whose physiological functions in the oral cavity have yet to be elucidated. In other portions of the gastrointestinal tract, PC is known to form a protective hydrophobic layer that is crucial for maintenance of the tissue's barrier properties against luminal insults such as acid and microbes. In contrast LysoPC (LPC) is injurious to biological membranes. The PC/LPC ratio has been used in pulmonary medicine and other fields as an index of conditions leading to membrane injury [3]. In the lung, the surface-activity of PC and related polar lipids are known to be increased in the presence of specific surfactant-association proteins (SPs) (notably SP-A, -B, -C and -D). It is known that lung tissue express and secrete a family of surfactant-associated proteins (SPs), which associate with PC and related SAPL to promote the

spreading and biological activity of these surfactant-lipids on the alveolar surface [4]. The hydrophobic SPs (SP-B & SP-C) directly act with SAPLs to enhance their surface-activity while the hydrophilic SPs (SP-A and SP-D) have critical roles regulating secretion and recycling of surfactant lipids and in innate immunity and protection against environmental insults [5],[6]. Our preliminary data showed that SPs are present in salivary gland. There are numerous oral symptoms that can occur in medically complex patients, which frequently is associated with smoking, taking certain medications (such as; anti-cholinergics, H-2 blockers anti-depressants and sympathetic agonists), chemotherapy or radiation therapy for head and neck or hematological cancers.

These symptoms can include local pain, hyposalivation, changes in saliva composition, dysphagia, taste alterations, and mucositis [7],[8]. Oral mucositis is perhaps the most severe oral side effect of cancer treatment and consists of painful inflammation and ulceration of the mucous membranes lining the oral cavity [9],[10]. However, patients taking multiple medications and medically compromised have shown to present similar clinical symptoms. It contributes to patient pain and discomfort and can lead to infections with viruses, bacteria or fungi, and in severe cases, systemic septicemia. Oral mucositis can make it difficult to eat and speak and cause weight loss. For many patients, severe oral mucositis becomes limiting on the dosage of xenogenic medications and chemotherapy, requiring treatment modifications and compromises to prognosis.

Objectives

Saliva contains surfactant phospholipids and surfactant-associated proteins, which gives saliva the ability to spread efficiently over the mucous membranes lining the oral cavity providing protection against a variety of insults [11]. Tobacco smoke provides may damage the mucus lining of the oral mucosa, in part because it increases temperature inside the mouth and burns tissues. In the clinical examination tobacco users have: hyperpigmentation, black hairy tongue, superficial glossitis, periodontitis, leucoedema, nicotinic stomatitis, leukoplakia or neoplasm [12]. Smoking leads to oral mucositis, which can be responsible for progress to oral cancer. Oral mucositis consists of painful inflammation and ulceration of the oral cavity resulting from reduced production and altered composition of saliva. We sought to determine the effects of smoking and gender on surfactant lipids and proteins in saliva to address the hypothesis that these activities reduce the composition of saliva of these critical and probable

protective factors, leading to altered saliva composition that may lack the protective properties necessary in the oral cavity. A finding of reduced PC and related SAPLs and SP concentration and/or output in saliva, or alternatively an increase in the levels of cytotoxic LPC and pro-apoptotic ceramides, will support our hypothesis of the significance of PC, related SAPLs, and SPs for oral health and suggest that supplying a form of exogenous PC, potentially, could be beneficial for maintaining oral health for these medically compromised patients. The saliva of smokers has shown a reduction in the content of PC, certain other SAPLs, and potentially an increase in the levels of cytotoxic LysoPC and pro-apoptotic ceramides. The possibility that the ratio of PC/LysoPC may be shifted in mucositis patients seems quite likely as the rate-limiting enzyme, phospholipase, in the conversion of PC to LysoPC is increased during periods of inflammation [3].

Methods

Saliva samples of patients presenting in Assessment Clinic at UTHealth School of Dentistry at Houston (UTSD) were collected after approval of IRB from smoking and non-smoking subjects. Samples snapped frozen upon collection for SP analysis for subsequent Western Blotting. Levels of sphingomyelin (Sph) phosphatidylcholine (PC) and lyso-PC (LPC) were determined by thin layer chromatography [13]. Levels of surfactant protein A (SP-A) were determined by ELISA (BioVendor Laboratory Medicine, Inc., cat #RD191139200R). Significance of the results was determined by the student's *t*-test.

Results

While changes in levels of LPC, Sph and PC differed very little between non-smokers and smokers, the PC/LPC ratio had a tendency to be lower in smokers compared to non-smokers. When the data was stratified by gender, there was no difference in levels of Sph and PC in saliva between male and female smokers, however, LPC levels were significantly higher in females smokers compared to male smokers, leading to a significant decreased PC/LPC ratio in female smokers. In addition, levels of SP-A were reduced in both male and female smokers compared to non-smokers, although significance was not met.

Conclusions

Smoking alters SP-A and phospholipid concentration, notably PC/LPC ratios in saliva, we also detected a smoking-related statistically significant decrease in the PC/LPC in female subjects that was not evident in males (Figure 1). SP-A levels were decreased in both men and

women who were smokers' vs non-smokers, which did not reach statistically difference (Figure 2). These results suggest that smoking alters the composition of saliva in a manner that may reduce protection of the oral cavity, which may explain why women smokers are at greater risk of developing oral mucositis. In the future, a finding of reduced PC and related SAPLs and SP concentration and/or output in saliva, or alternatively an increase in the levels of cytotoxic LPC and pro-apoptotic ceramides (e.g. SM), will support our hypothesis of the significance of PC, related SAPLs, and SPs for oral health, and suggest that surfactant-replacement strategy could be beneficial for maintaining oral health of medically compromised patients.

Figure 1: Salivary phospholipid (female smoker vs. non-smoker).

Figure 2: SP-A levels in saliva of all gender non-smokers and smokers.

Acknowledgement

Many thanks to the Department of Integrative Biology and Pharmacology and Department of General Practice and Dental Public Health at UTHHealth for their support of this project. Also, thanks to Mr. Davor Seferovic for his assistance in collection of samples. Supported by NIH grant R21 CA202751.

References

1. <http://www.aaom.com/index.php?option=com>
2. <http://www.cancer.gov/about-cancer/treatment/side-effect/mouth-troat/oral-complications-hp-pdg>
3. Slomiany BL, Zdebska E, Murty VLN, Slomiany A, Petropoulou K, Mandel ID. Lipid composition of human labial salivary gland secretions. *Archives of Oral Biology*. 1983; 28:711-4. DOI: [http://dx.doi.org/10.1016/0003-9969\(83\)90105-x](http://dx.doi.org/10.1016/0003-9969(83)90105-x)
4. Haagsman HP, Diemel RV. Surfactant-associated proteins: functions and structural variation. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*. 2001; 129:91-108. DOI: [http://dx.doi.org/10.1016/s1095-6433\(01\)00308-7](http://dx.doi.org/10.1016/s1095-6433(01)00308-7)
5. Crouch E, Wright JR. Surfactant proteins A and D and pulmonary host defense. *Annual Review of Physiology*. 2001; 63:521-54. DOI:<http://dx.doi.org/10.1146/annurev.physiol.63.1.521>
6. LeVine AM, Whitsett JA. Pulmonary collectins and innate host defense of the lung. *Microbes and Infection*. 2001; 3:161-6. DOI: [http://dx.doi.org/10.1016/s1286-4579\(00\)01363-0](http://dx.doi.org/10.1016/s1286-4579(00)01363-0)
7. Baer AN, Walitt B. Sjögren syndrome and other causes of sicca in older adults. *Clinics in Geriatric Medicine*. 2017; 33:87-103. DOI:<http://dx.doi.org/10.1016/j.cger.2016.08.007>.
8. Bostock C, McDonald C. Antimuscarinics in older people: dry mouth and beyond. *Dental Update*. 2016; 43:186-91. DOI:<http://dx.doi.org/10.12968/denu.2016.43.2.186>.
9. Öhrn K, Wahlin Y-B, Sjöden P-O. Oral status during radiotherapy and chemotherapy: a descriptive study of patient experiences and the occurrence of oral complications. *Supportive Care in Cancer*. 2001; 9:247-57. DOI:<http://dx.doi.org/10.1007/s005200000214>.
10. Vissink A, Jansma J, Spijkervet FKL, Burlage FR, Coppes RP. Oral sequelae of head and neck radiotherapy. *Critical Reviews in Oral Biology & Medicine*. 2003; 14:199-212. DOI: <http://dx.doi.org/10.1177/154411130301400305>.

11. Hall HD. Protective and maintenance functions of human saliva. *Quintessence Int.* 1993 Nov; 24(11):813-6. PMID: <https://www.ncbi.nlm.nih.gov/pubmed/20830897>
12. Michalak E, Halko-Gąsior A, Chomyszyn-Gajewska M. *Przegl Lek.* 2016; 73(7):516-9. Review. Polish. PMID: <https://www.ncbi.nlm.nih.gov/pubmed/29677424>
13. Zayat M, Lichtenberger LM, Dial EJ. Pathophysiology of LPS-induced gastrointestinal injury in the rat: role of secretory phospholipase A2. *Shock.* 2007; 1. DOI:<http://dx.doi.org/10.1097/shk.0b013e318160f47f1p6>

Copyright: © **Adibi et al.** This is an Open Access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.